I. Molecular mechanisms of peroxisome mutants, which were defective in Autophagy-related 2, mutants in concert with the analyses of peup1 functional transformation of peroxisomes using these apem1, apem3, apem4, apem9 and apem10 mutants. From these analyses, we will be able to identify the components responsible for peroxisome biogenesis, functions and maintenance, and to address the mechanism at the molecular level.

II. Accumulation mechanism of seed storage oils and proteins

Plant seeds accumulate huge amounts of storage reserves such as oils, carbohydrates and proteins. Humans use these storage reserves in food and industrial materials. Storage reserves vary among different types of plant seeds. Wheat, maize and rice seeds mainly accumulate starch, whereas rapeseed, pumpkin and sesame contain large amounts of oils. Soybeans contain proteins as a major reserve. Storage oils and storage proteins are synthesized in the endoplasmic reticulum (ER) and accumulated in oil bodies and protein bodies, respectively, during the same period of seed development (Figure 2).

We are analyzing the molecular mechanisms controlling oil and protein contents in seeds. Based on the analysis of the temporal sequence of oil and protein synthesis during seed development in Arabidopsis thaliana, which produces seeds containing approximately 30% oil and 30% protein, we...
revealed that the extension of *WRINKLED1* (*WRI1*), a transcription factor in fatty acid biosynthesis, expression during the midphase of seed development significantly enhanced seed oil content, and caused an enlargement of seed size.

We are also currently investigating the mechanisms of oil accumulation in other plant species. In addition, we are trying to apply our knowledge and techniques to increase beneficial storage reserves.

### III. Development of Gateway-technology vectors for plant research

Gateway cloning is a popular technology, which allows the simultaneous generation of multiple constructs containing a range of fusion genes. We have developed various types of Gateway cloning-compatible vectors for the improvement of resources in the plant research field. As of writing, we have provided vector sets to detect multiple protein-protein interactions in vivo using multi-color bimolecular fluorescence complementation, and the binary vectors to facilitate tripartite DNA assembly and promoter analysis with various reporters and tags in the liverwort *Marchantia polymorpha* (Figure 3). We will continue developing other useful Gateway cloning-compatible vectors to contribute to the plant research community.

### IV. Construction of The Plant Organelles Database 3 (PODB3) and Plant Organelles World

The Plant Organelles Database 3 (PODB3) was built to promote a comprehensive understanding of organelle dynamics. This public database is open to all researchers. PODB3 consists of six individual units: the electron micrograph database, the perceptive organelles database, the organelles movie database, the functional analysis database, and external links. The function of each database is as follows:

- The electron micrograph database provides information on the ultrastructures in plant cells.
- The perceptive organelles database shows organelle dynamics responding to environmental stimuli.
- The organelles movie database contains time-lapse images and 3D structure rotations.
- The organelome database is a compilation of static image data of various tissues of several plant species at different developmental stages.
- The functional analysis database is a collection of protocols for plant organelle research.

Through these databases, users can easily grasp plant organelle dynamics. Plant Organelles World, which is built based on PODB3, is an educational tool for engaging members of the non-scientific community to explore plant biology. We hope that both PODB3 and Plant Organelles World are of help to researchers as well as the general public.

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**Figure 3.** R4pMpGWB and R4L1pMpGWB systems for construction of fusion genes in *M. polymorpha*. (A) In the R4pMpGWB system, promoter and cDNA entry clones and R4pMpGWB vectors are used in a tripartite LR reaction to form a C-terminal fusion of cDNA-encoded protein and a reporter or tag. (B) Thallus epidermal cells expressing the peroxisome-targeted Citrine under the 35S promoter. (C) Sperm cell expressing Lifeact-Venus under the endogenous *PROTAMINE* promoter Bars: 10 μm for (B) and 2 μm for (C). (D) In the R4L1pMpGWB system, the promoter entry clones and R4L1pMpGWB vectors are used for a bipartite LR reaction. (E) GUS staining in thalli expressing *proMgEF1α::GUS*. (F) Luminescence images after heat shock of transgenic plants bearing *proMgHSP17.8A1::ELuc(PEST)*. Bars: 1 mm

**Publication List:**

- *Original paper*

- *Review article*